



Appendix of Pending Claims

TECH CENTER 1600/2800

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1. (Amended) A method of identifying a sequence alteration in a target nucleic acid as compared to a control nucleic acid, said method comprising:

a) hybridizing a plurality of nucleic acid probes with said target nucleic acid, wherein said probes are complementary to different overlapping regions of said control nucleic acid;

b) determining the melting temperature (T_m) of at least two overlapping probes from said target nucleic acid;

c) determining the ΔT_m for each of said overlapping probes, wherein the ΔT_m is the difference between the T_m of said target nucleic acid and one of said overlapping probes and the T_m of said control nucleic acid and the same overlapping probe; and

d) determining the difference, if any, in the ΔT_m of at least two overlapping probes as an indication of the presence or absence of a sequence alteration in said target nucleic acid as compared to said control nucleic acid.

2. The method of Claim 1, wherein the difference in ΔT_m between at least two overlapping probes indicates the location of a nucleotide difference in the target nucleic acid as compared to the control nucleic acid.

3. The method of Claim 1, wherein the difference in ΔT_m between at least two overlapping probes indicates a substitution in the target nucleic acid sequence as compared to the control nucleic acid.

4. The method of Claim 3, wherein the difference in ΔT_m between at least two overlapping probes indicates the type of nucleotide substituted in the target nucleic acid sequence as compared to the control nucleic acid.

5. The method of Claim 3 or 4, wherein the difference in ΔT_m between at least two overlapping probes indicates the location of the substitution in the target nucleic acid sequence as compared to the control nucleic acid.

6. A method of identifying a sequence alteration in a target nucleic acid as compared to a control nucleic acid, said method comprising:

54B
D1
COU⁴,
a) hybridizing a plurality of nucleic acid probes with said target nucleic acid, wherein a first set of probes is complementary to regions of said control nucleic acid separated by one or more nucleotides and at least a second set of probes is complementary to regions of said control separated by one or more nucleotides, wherein the regions complementary to said second set of probes include the nucleic acids separating the first set of probes and are overlapping with the regions complementary to said first set of probes;

b) determining the melting temperature (T_m) of at least two overlapping probes from said target nucleic acid;

C1
COU⁴,
c) determining the ΔT_m for each of said overlapping probes, wherein the ΔT_m is the difference between the T_m of said target nucleic acid and one of said overlapping probes and the T_m of said control nucleic acid and that same probe; and

d) determining the difference in ΔT_m , if any, of at least two overlapping probes as an indication of the presence or absence of a sequence alteration in said target nucleic acid as compared to said control nucleic acid.

6. (Amended) A method of identifying a sequence alteration in a target nucleic acid as compared to a control nucleic acid, said method comprising:

a) hybridizing a plurality of nucleic acid probes with said target nucleic acid, wherein a first set of probes is complementary to regions of said control nucleic acid separated by one or more nucleotides and at least a second set of probes is complementary to regions of said control separated by one or more nucleotides, wherein the regions complementary to said second set of probes include the nucleic acids separating the first set of probes and are overlapping with the regions complementary to said first set of probes;

b) determining the melting temperature (T_m) of at least two overlapping probes from said target nucleic acid;

c) determining the ΔT_m for each of said overlapping probes, wherein the ΔT_m is the difference between the T_m of said target nucleic acid and one of said overlapping probes and the T_m of said control nucleic acid and the same overlapping probe; and

d) determining the difference in ΔT_m , if any, of at least two overlapping probes as an indication of the presence or absence of a sequence alteration in said target nucleic acid as compared to said control nucleic acid.

7. The method of Claim 6, wherein only two sets of probes are used.

8. The method of Claim 6, wherein the difference in ΔT_m between at least two overlapping probes indicates the location in the control nucleic acid of a nucleotide difference between the target nucleic acid and the control nucleic acid.

9. The method of Claim 6, wherein the difference in ΔT_m between at least two overlapping probes indicates a substitution in the sequence of the control nucleic acid.

10. The method of Claim 9, wherein the difference in ΔT_m between at least two overlapping probes indicates the type of nucleotide substituted in the sequence of the control nucleic acid.

11. The method of Claim 9 or 10, wherein the difference in ΔT_m between at least two overlapping probes indicates the location of the substitution in the sequence of the control nucleic acid.

12. The method of Claim 6, wherein ΔT_m is determined for at least two probes of said first set of probes which are complementary to adjacent regions of said control nucleic acid and at least one probe of a second set of probes which overlaps with each of said at least two probes of said first set of probes.

13. The method of Claim 12, wherein a ΔT_m of zero for said at least two probes of said first set of probes and a ΔT_m of greater zero for said at least one probe of a second set of probes indicates the location of a sequence alteration in the target nucleic acid as compared to the control nucleic acid at a nucleotide in the control nucleic acid separating the regions to which said at least two probes of said first set of probes are complementary.

14. The method of Claim 13, wherein the regions of said control to which said first set of probes is complementary are separated by a single nucleotide and the location of said sequence alteration is at said single nucleotide.

15. The method of Claim 14, wherein the ΔT_m of said at least one probe of a second set of probes indicates a substitution in the sequence of the control nucleic acid.

16. The method of Claim 15, wherein the ΔT_m of said at least one probe of a second set of probes indicates the type of nucleotide substituted in the sequence of the control nucleic acid.